

AMENDMENT

In the Specification:

Please make the following amendments in the specification. Pursuant to 37 C.F.R. §1.121(b)(1)(iii), a marked-up copy of the amended paragraphs in the specification is attached herewith on separate pages.

Please replace paragraph 1 on page 7 with the following:

--FIG. 1 and 2 show the native DNA sequences encoding, and the deduced amino acid sequences of, human basic FGF (SEQ ID NO: 1) and acidic FGF (SEQ ID NO: 3), respectively.--

[Please replace paragraph 2 on page 7 with the following:]

B
--FIG. 3 shows a comparison of the amino acid sequences for human acidic (SEQ ID NO: 4) and basic (SEQ ID NO: 2) FGF and the various regions targeted for alteration, including potential heparin-binding domains and receptor-binding regions.--

[Please replace paragraph 3 on page 7 with the following:]

--FIG. 4 shows the construction of a synthetic tryptophan operon promoter and operator regulatory sequence (SEQ ID NOS: 5-6), and a restriction site map of plasmid pTRP-233. --

Please replace the last paragraph on page 39, which continues on page 40, with the following:

B
--There are only two amino acid differences between basic bovine FGF and human FGF, at position 123, where the bovine protein has Ser and the human protein has Thr, and at position 137, where the bovine protein has Pro and the human has Ser. These differences are the result of a single nucleotide difference in each case; therefore bovine cDNA may conveniently be modified by site directed mutagenesis as described below to encode the human protein, and, indeed, standard site-specific mutagenesis techniques were used to

alter these codons. The lambda BB2 clone (ATCC No. 40196) was digested with EcoRI and the 1.4 kb region spanning the bFGF protein-encoding portion was ligated into the EcoRI site of M13mp8 and phage carrying the insert in the correct orientation were recovered. The in vitro mutagenesis was carried out in the presence of three oligonucleotides: the "universal" primer, a 17-mer; the mutagenic 16-mer 5'-GAAATACACCAGTTGG-3' (SEQ ID NO: 7); which alters the coding sequence at codon 123, and the mutagenic 17-mer 5'-ACTTGGATCCAAAACAG-3 (SEQ ID NO: 8), which alters the sequence at codon 137. The mutagenized phage was also subjected to a second round of in vitro primer-directed mutagenesis to create a HindIII site 34 bp downstream from the translation termination codon using the mutagenic 25-mer, 5'-TTTTACATGAAGCTTATATTCAG-3' (SEQ ID NO: 9). The resultant mutated DNA was sequenced by dideoxynucleotide sequence analysis (Sanger et al, supra) to confirm that the desired mutagenesis had occurred, and the approximately 630 bp fragment spanning the FGF coding region was excised with HindIII and ligated into HindIII digested pUC13 to obtain the intermediate plasmid pJJ15-1.

Please replace the table on the bottom of page 40 (extending onto page 41) with the following:

Number	Sequence	
1670	5'-pAGCTTCATATGGCTGCTGGTTCTATCACTACC	(SEQ ID NO:10)
1623R	5'-pCTGCCAGCTCTGCCAGAACGACGGTGGTT	(SEQ ID NO:11)
1624R	5'-pCTGGTGCCTTCCCACCAGGTCACTCAA	(SEQ ID NO:12)
1625R	5'-pAGACCCAAAACGTCTGTACTGAAAAAC	(SEQ ID NO:13)
1680	5'-pGGTGGTTCTCCTGCGCA	(SEQ ID NO:14)
1679	5'-pTAGAACCAAGCAGCCATATGA	(SEQ ID NO:15)
1622	5'-pTCTTCTGGCAGAGCTGGCAGGGTGA	(SEQ ID NO:16)

B3
1619 5'-pACCTGGTGGGAAGGCACCAGAACCG (SEQ ID NO:17)
1626 5'-pAGTACAGACGTTGGTCTTGAGTG (SEQ ID NO:18)
1673 5'-pAGCTTGCAGGAAGAAACCACCGTTTGC (SEQ ID NO: 19)

Please replace the paragraph starting at page 41, line 5, with the following:

B4
Construction of Synthetic Gene for the Amino Terminal Region of bFGF (SEQ ID NOS: 20-21):

Please replace the first complete paragraph on page 46 with the following:

B5
In this example, cysteine residues at positions 34 and 101 of the human basic FGF protein were changed to serine residues thereby producing a double mutation. Approximately 2 micrograms each of the mutagenic 23-mer 5'-ACGTCTGTACTCCAAAAACGGTG-3' (SEQ ID NO: 22) (#2222); which alters the sequence at codon 34, and the mutagenic 23-mer 5'-TACAGACGAGTCTTCTTTTG-3' (SEQ ID NO: 23) (#2323); which alters the sequence at codon 101 were incubated in 50 ul of 1.times.kinase/ligase buffer (7 mm Tris-HCl pH 7.6, 10 mm MgCl₂, 5 mm dithiothreitol) with 1 mM ATP and 5 units T₄ polynucleotide kinase for 30 minutes at 37.degree. C. The phosphorylated oligonucleotides were diluted two-fold into 1 mM Tris-HCl, pH 8.0 and 1 mM EDTA.

Please replace Table 1 on page 48 with the following:

TABLE 1

<u>bFGF Analog⁺</u>	<u>Oligonucleotide[#]</u>	<u>Number*</u>
1) bFGF-C78S	5'-pCAAAGGAGTGTCTGCAAACCGTT (SEQ ID NO:24)	2217
2) bFGF-C96S	5'-pAGCTTCTAAATCTGTTACAGACG (SEQ ID NO:25)	2218

3) bFGF-C78/96S 2218/2217
4) bFGF-C34/78/ 2217/2218/
96/101S 2222/2323
25 5'-pACGTCTGTACTCCAAAAACGGTG (SEQ ID NO:26) 2222
5'-pTACAGACGAGTCTTCTTTTG (SEQ ID NO:27) 2323
5) bFGF-C34/78/96S
2222/2218/2217
6) bFGF-C78/96/101S 2217/2218/2323
7) bFGF-C34/78/101S 2222/2217/2323
8) bFGF-C34/78S 2222/2217
9) bFGF-C34/101S 2222/2323--

Please replace the table in Example 9 (pages 54-55) with the following:

<u>Analog</u>	<u>Oligonucleotide</u>	<u>Number</u>
bFGF-K35S	5'-pGTCTGTACTGCTCAAACGGTGGTT (SEQ ID NO:28)	2553
bFGF-R42L	5'-pTTTCTTCCTGCTCATCCACCCCG (SEQ ID NO:29)	2327
bFGF-D46A	5'-pCATCCACCCGCCGGCGAGTGG (SEQ ID NO:30)	2221
bFGF-R48L	5'-pCCCCGACGGCCTAGTGGACGGGG (SEQ ID NO:31)	2454
bFGF-R48A	5'-pACCCCGACGGCGCAGTGGACGGGG (SEQ ID NO:32)	2555
bFGF-D50A	5'-pCGGCCGGAGTGGCCGGGTCCGCG (SEQ ID NO:33)	2224
bFGF-V52K	5'-pGAGTGGACGGAAACGCGAGAAGAG (SEQ ID NO:34)	2491
bFGF-R53L	5'-pGGACGGGGTCCTCGAGAAGAGCG (SEQ ID NO:35)	2220
bFGF-K55M	5'-pGGTCCCGCGAGATGAGCGACCCAC (SEQ ID NO:36)	2223

bFGF-K55I	5'-pGGTCCCGAGATAAGCGACCCACA (SEQ ID NO:37)	2567
bFGF-D57A	5'-pCGAGAACAGCGCCCCACACATCA (SEQ ID NO:38)	2225
bFGF-H59N	5'-pGAGCGACCCAAACATCAAACATAC (SEQ ID NO:39)	2383
bFGF-R9OT	5'-pAGAACATGGAACCTTACTAGCTTC (SEQ ID NO:40)	3088
bFGF-D99A	5'-pATGTGTTACAGCAGAGTGTCT (SEQ ID NO:41)	2381
bFGF-E100A	5'-pGTTACAGACGCCTGTTCTTTTG (SEQ ID NO:42)	2549
bFGF-E100S	5'-pGTGTTACAGACAGTTGTTCTTTT (SEQ ID NO:43)	2380
bFGF-E105S	5'-pGTTCTTTTCACGATTGGAGT (SEQ ID NO:44)	2556
bFGF-R106L	5'-pCTTTTGAACTATTGGAGTCTA (SEQ ID NO:45)	2494
bFGF-E108A	5'-pTGAACGATTGGCATCTAATAACTA (SEQ ID NO:46)	2554
bFGF-Y112A	5'-pAGTCTAATAACGCAAATACTTACCG (SEQ ID NO:47)	2450
bFGF-N113S	5'-pCTAATAACTACAGTACTTACCGG (SEQ ID NO:48)	2452
bFGF-R116T	5'-pCAATACTTACACTCAAGGAAATA (SEQ ID NO:49)	3091
bFGF-R118L	5'-pCAATACTTACCTGTCAAGGAAAT (SEQ ID NO:50)	2483
bFGF-K119S	5'-pACCGGTCAAGGTCTACACCAGTTG (SEQ ID NO:51)	2548
bFGF-(41-43)	5'-pGGTGGTTCTTCCACCCCGACGGC (SEQ ID NO:52)	2336
bFGF-(49-51)	5'-pCCCGACGGCCGAGTCCCGAGAG (SEQ ID NO:53)	2335
bFGF-(62-64)	5'-pCCACACATCAAACAAGCAGAAGAG (SEQ ID NO:54)	2334
bFGF-(83-85)	5'-pGCAAACCGTTACAAAGAAGATGGA (SEQ ID NO:55)	2333
bFGF-(105-107)	5'-pTGTTCTTTTGAGTCTAATAAC (SEQ ID NO:56)	2332
bFGF-(112-114)	5'-pGAGTCTAATAACTACCGGTCAAGG (SEQ ID NO:57)	2337-7

Please replace the table in Example 10 (page 61) with the following:

<u>Analog</u>	<u>Oligonucleotide</u>	<u>Number</u>
bFGF-K27M	5'-pAGGTCACTTCATGGACCCAAAACG (SEQ ID NO:58)	2487
bFGF-K30A	5'-pTCAAAGACCCAGCACGTCTGTACT (SEQ ID NO:59)	2566
bFGF-R31S	5'-pAAGACCCAAAATCTCTGTACTGCA (SEQ ID NO:60)	2568
bFGF-D28K	5'-pGTCACTTCAAAAGCCAAAACGTCT (SEQ ID NO:61)	2480
bFGF-R118L	5'-pCAATACTTACCTGTCAAGGAAAT (SEQ ID NO:62)	2483
bFGF-K35S	5'-pGTCTGTACTGCTCAAACGGTGGTT (SEQ ID NO:63)	2553
bFGF-K128S	5'-pATGTGGCACTGGAGCGAACTGGCA (SEQ ID NO:64)	2545
bFGF-K128E	5'-pATGTGGCACTGGAGCGAACTGGCCA (SEQ ID NO:65)	3332
bFGF-R129T	5'-pGGCACTGAAAACACTGGGCAGT (SEQ ID NO:66)	3087
bFGF-k128E/R129T		
bFGF-K134S	5'-pCTGGGCAGTATTCTCTTGGATCCAA (SEQ ID NO:67)	3212
bFGF-K138S	5'-pAACTGGATCCTCTACAGGACCTGG (SEQ ID NO:68)	3215-

Please replace paragraph 1 on page 62 with the following:

B8
The blunted NdeI-HindIII FGF fragment from pUC9delH3-PTSF-3 was subcloned into the SmaI-HindIII site of M13mp18. An oligo was used to introduce a new NdeI site in the FGF molecule at amino acid 25 using in vitro mutagenesis. The new NdeI site serves as both a new restriction site for subcloning the FGF fragment and also as a new translational start site for the shortened form of FGF. The mutagenic oligo used has the sequence:

B9
5'-TTG GGT CTT TGA AGT GCA TAT GTG GGA AGG CAC CAG (SEQ ID NO: 69)--